

SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL
SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> PCA40739/HMY

<150> KR1020030072216

<151> 2003-10-16

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 1

gggaagcttc gatcggacat ccagatgacc cagtctccat cctccctgtc tgcatctgta 60

ggggacagag tcacc 75

<210> 2

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 2

tggtttttgc tgataccagg ctaagtaatt totgatgcc tgacttgccc gacaagtgat 60

ggtgactctg tcccctacag

80

<210> 3
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of light chain variable region

<400> 3
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tgcaatcagg ggtcccatct 80

<210> 4
<211> 80
<212> DNA
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<220>
<223> gene fragment of light chain variable region

<400> 4
aggctgtagg ctgctgatgg tgagagtga atctgtccca gatccaçtgc cactgaaccg 60
agatgggacc cctgattgca 80

<210> 5
<211> 80
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<220>
<223> gene fragment of light chain variable region

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<400> 5
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caccgatac ttttggccag 80

<210> 6
<211> 41
<212> DNA
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<400> 6
tttgatttcc accttgggtcc cctggccaaa agtatacggg g 41

<210> 7
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 7
gggaagcttc gatcggaggt gcagctggtg gaggcttggg gaggcttggg acagcccggc 60
aggtccctga gactc 75

<210> 8
<211> 79
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

4

<400> 8
agcttgccgg acccagtgca tggcataatc atcaaaggtg aatccagagg ccgcacagga 60
gagtctcagg gacctgccg 79

<210> 9
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 9
tgcactgggt ccggcaagct ccaggaagg gcctggaatg ggtctcagct atcacttgga 60
atagtgtca catagactat 80

<210> 10
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 10
atacaggag ttcttggcgt tgtctctgga gatggtgaat cggccctcca cagagtccgc 60
atagtctatg tgaccactat 80

<210> 11
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 11
acgccaagaa ctccctgtat ctgcaaatga acagtctgag agctgaggat acggccgtat 60
attactgtgc gaaagtctcg 80

<210> 12
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 12
cactcgagac ggtgaccagg gtaccttggc cccaatagtc aagggaggac gcggtgctaa 60
ggtacgagac tttcgcacag taat 84

<210> 13
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR forward primer specific for heavy chain

<400> 13
cccaagctta ggctccacc aagggcccat cgggtcttcc 39

<210> 14
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for heavy chain

<400> 14
gggggatcct tatgggcacg gtgggcatgt gtgagttttg tcacaaga 48

<210> 15
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR forward primer specific for light chain

<400> 15
cccaagcttt cgcgaactgt ggctgcacca tctgtcttca tc 42

<210> 16
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for light chain

<400> 16
cccgatccc taacactctc ccctgttgaa gctctttgtg ac 42

<210> 17
<211> 69
<212> DNA
<213> modified E. coli thermostable enterotoxin II signal sequence

<400> 17
atgaaaaaga caatcgcat tcttcttgca tctatgttcg ttttttctat tgctacaaat 60
gcccaggcg 69

7

<210> 18
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing *Stu*I restriction enzyme site

<400> 18
tctattgcta caaatgccca ggccttccca accattocct tatcc 45

<210> 19
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing *Stu*I restriction enzyme site

<400> 19
agataacgat gtttacgggt ccggaagggt tggttaagga atagg 45

<210> 20
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer specific for light chain

<400> 20
gggggatcct cacgcggcgc atgtgtgagt ttgtcacaa gatttaggct c 51

<210> 21
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21
gggggatcca ggaggtgatt tatgaaaag acaatcgcat ttc 43

<210> 22
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing BpuI restriction enzyme site

<400> 22
ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca ttic 44

<210> 23
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing BpuI restriction enzyme site

<400> 23
ggggctcagc tcacgcggcg catgtgtgag ttttgcaca agatttaggc tc 52

<210> 24
<211> 63
<212> DNA
<213> E. coli OmpA signal sequence

<400> 24
atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa 60

gct 63

<210> 25
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer specific for heavy chain

<400> 25
gaggttcagc tagtcgagtc aggaggcggt 30

<210> 26
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing HindIII and StuI restriction enzyme sites

<400> 26
gggagatctt cacgcggcgc atgtgtgagt ttgtgcacaa gatttaggct c 51

<210> 27
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 27
gacattcaaa tgaccagag cccatccagc 30

<210> 28

10

<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing HindIII and NruI restriction enzyme sites

<400> 28
cccagatctc taacactctc cctgttgaa gctctttgtg ac 42

<210> 29
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29
ggggtcgaca ggaggtgatt tatgaaaaag acagctatcg c 41

<210> 30
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing SalI restriction enzyme site

<400> 30
ggggtcgact cagcggcgc atgtgtgagt ttgtcacaa gatttaggct c 51

<210> 31
<211> 42
<212> DNA
<213> Artificial Sequence

11

<220>

<223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site

<400> 31

gggcataatga aaaagacaat cgcatttctt cttgcatcta tg

42

<210> 32

<211> 705

<212> DNA

<213> Artificial Sequence

<220>

<223> TNF-alpha heavy chain

<400> 32

gaggttcagc tagtcgagtc aggaggcggg ttggtacagc ccggcaggtc cctgagactc 60

tcctgtgcgg cctctggatt cacctttgat gattatgccat tgcactgggt ccggcaagct 120

ccaggaagg gcctggaatg ggtctcagct atcacttggga atagtgggtca catagactat 180

gaggactctg tggaggggccg attcaccatc tccagagaca acgccaagaa ctccctgtat 240

ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg 300

taccttagca ccgcgtcctc ccttgactat tggggccaag gtaccctggt caccgtctcg 360

agtgcctcca ccaagggccc atcgggtctc cccctggcac cctcctccaa gagcacctct 420

gggggcacag oggcccctggg ctgcctgggtc aaggactact tccccgaacc ggtgacgggtg 480

tcgtggaact caggcgcctt gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540

tcaggactct actccctcag cagcgtgggtg accgtgccct ccagcagctt gggcaccag 600

acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660

cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc catag 705

12

<210> 33
 <211> 645
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> TNF-alpha light chain

<400> 33
 gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtagggga cagagtcacc 60
 atcacttgtc gggcaagtca gggcatcaga aattacttag cctggatatca gcaaaaacca 120
 gggaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg ggtcccatct 180
 cggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag cctacagcct 240
 gaagatgttg caacttatta ctgtcaaagg tataaccgtg caccgtatac ttttggccag 300
 gggaccaagg tggaaatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 360
 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420
 cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag 480
 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540
 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600
 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag 645

<210> 34
 <211> 7
 <212> PRT
 <213> TNF-alpha light chain

<400> 34
 Asp Ile Gln Met Thr Gln Ser
 1 5

13

<210> 35
<211> 8
<212> PRT
<213> TNF-alpha heavy chain

<400> 35
Glu Val Gln Leu Glu Val Asp Ser
1 5

<210> 36
<211> 12
<212> PRT
<213> N-terminal sequence of recombinant TNF-alpha

<400> 36
Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser
1 5 10